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Activated Sludge Biodegradation of 12 Commercial Phthalate Esters

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The activated sludge biodegradability of 12 commercial phthalate esters was evaluated in two test systems: (i) a semicontinuous activated sludge test and (ii) an acclimated 19-day die-away procedure. Both procedures demonstrated that phthalate esters are rapidly biodegraded under activated sludge conditions when loss of the parent phthalate ester (primary degradation) is measured.

The biodegradability potential of a broad range of commercial phthalate esters (PAEs) has previously been established (5), using a CO_2 evolution screening test. However, the PAE biodegradability in activated sludge systems that model a wastewater treatment plant has only been examined for a limited number of PAE compounds (3, 6). To determine the biodegradability of these compounds under activated sludge treatment conditions, 12 PAE composite samples representative of major commercial PAE products were tested by the semicontinuous activated sludge (SCAS) test recommended by the Soap and Detergent Association (4) followed by a 19-day die-away procedure recommended by the U. S. Environmental Protection Agency (7), using the acclimated sludge from the SCAS procedure.

General method. An SCAS (4) procedure was used for acclimation and to determine 24-h biodegradation, and the acclimated mixed culture from the SCAS procedure was used in a 19-day die-away test (7). The procedure was initially designed to be used with chemicals that were water soluble enough so that dissolved organic carbon could be used to determine percent removal. Because of the low water solubility of some of the PAEs, the method was modified to allow for direct chemical analysis of the PAE.

Besides biodegradation, volatilization and adsorption may be important removal processes in SCAS units, and these processes need to be eliminated to demonstrate biodegradation. Volatilization was not considered important in the SCAS units because five PAEs, including four PAEs used in this study (see Table 1), were tested for volatilization from SCAS units by Saeger and Tucker (3). They examined the off-gases from the SCAS units which were passed through three hexane scrubbers. Less than 0.5% volatility loss per 24 h was observed for the PAEs. Henry's Law constant (air/water equilibrium constant) for most of the PAEs can be calculated from the water solubility and vapor pressure measurements of Howard et al. (P. H. Howard, S. Banerjee, and K. H. Robillard, Environ. Toxicol. Chem., in press). These indicated that the rest of the PAEs should not be any more volatile than the five PAEs measured by Saeger and Tucker (3). This includes both dimethyl and diethyl phthalates which have higher vapor pressures but are considerably more water soluble than the other PAEs. Adsorption to sludge or glassware was not considered to be a problem because the concentration of PAE measured in 50-ml aliquots taken from the SCAS units within several hours after addition during either the draw-and-fill period or the 19-day die-away were very close to the nominal concentration, indicating that the PAE was evenly distributed throughout the SCAS units and not adsorbed on the glassware. Since aliquots of mixed activated sludge were analyzed periodically to determine biodegradation, any PAE adsorbed to sludge would have been analyzed and not counted as biodegradation. Therefore, since adsorption to glassware or to sludge is eliminated as removal processes, the loss measured by the analytical procedure is due to biodegradation.

Daily operation of the SCAS unit was identical to the Soap and Detergent Association procedure except the total volume was 2 liters instead of 1.5 liters. After the sludge was acclimated to the synthetic sewage feed (4) and the dissolved organic carbon removal efficiency remained at >70%, PAE and diethylene glycol feeding began, and then the draw-and-fill procedure was maintained over a 3-week period. Each PAE was added to a single unit, using an acetone stock solution (<200μl of acetone added) to provide a concentration of 1 mg/liter during week 1 and 3 mg/liter during weeks 2 and 3. When an acetone stock solution was not used, dispersion in the unit was very poor as determined by sample aliquots. During the 3-week draw-and-fill phase, 24-h SCAS PAE biodegradation was determined once each week in the middle of the week by analyzing a 50-ml aliquot from each unit after feeding of PAE and synthetic sewage and after a 23-h aeration period. The difference in concentration for the two aliquots was reported as percent biodegradation. At the beginning of week 3, the single unit was split into two units and the 24-h biodegradation in each unit was determined in the middle of the week. Dissolved organic carbon was measured in all units including the diethylene glycol control.

After the 3-week draw-and-fill period, the suspended solids in each of the two SCAS units were adjusted to 1,000 mg per unit (500 mg/liter), and enough PAE was added to bring the concentration to 3 mg/liter in the two units at the beginning of the 19-day die-away. Aeration was maintained and samples were taken on days 0, 1, 2, 3, 4, 5, 9, 12, 15, and 19 or until >90% degradation was observed on two consecutive sampling days.

Chemicals. Commercial samples of 12 PAEs (see Table 1) were supplied by U.S. manufacturers for testing. If more than one manufacturer produced the individual PAE, the PAE product provided was an equal-proportion blend. Before testing, the PAEs were analyzed to confirm that they were within commercial specification limits.

Analytical procedure. The extraction and analysis procedures of Saeger and Tucker (3) were used. Standard addi-

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TABLE 1. Comparison of PAE biodegradability in activated sludge systems

PAE	Code	% Degraded		Initial concn (mg/liter)	Activated sludge die-away, 1st order rate constant × 10^2 (units not given)[c]	Semicontinuous activated sludge, 24 h [% mean degradation (±SE)][d]	Activated sludge die-away (days to achieve ≥90% biodegradation)[e]
		Activated sludge, 48 h[a]	Semicontinuous activated sludge, 24 h[b]				
Dimethyl	DMP				9.49 ± 0.41	>81.0 (11.1)	1, 1
Diethyl	DEP				6.59 ± 0.43	>94.8 (1.6)	1, 1
Di-*n*-propyl	DPP				10.71 ± 0.73		
Di-*n*-butyl	DBP				13.26 ± 0.73	>95.0 (1.7)	1, 1
Di-*n*-pentyl	DPenP				9.12 ± 1.51		
Butyl benzyl	BBP	99	93 ± 6	3.3			
			99+	133			
Butyl 2-ethylhexyl	BOP					84.5 (1.2)	3, 3
Butylglycol butyl	BGBP		99+	3.3–133			
Dihexyl	DHP				6.86 ± 0.23	>92.8 (2.3)	1, 1
Di-*n*-heptyl	DHepP				7.18 ± 0.50		
n-Hexyl, *n*-octyl, *n*-decyl	610P					59.8 (4.2)	4, 4
Di-*n*-octyl	DNOP				1.57 ± 0.17		
Diisooctyl	DIOP					84.5 (4.6)	4, 4
Di-2-ethylhexyl	DEHP	91	70 ± 11	3.3		81.5 (4.3)	2, 5
			78 ± 3	3.3			
Diisononyl	DINP					67.8 (7.2)	5, 5
Heptyl, nonyl, undecyl	711P		52 ± 10	3.3		65.0 (5.1)	3, 4
			54 ± 7	13.3			
Diisodecyl	DIDP					68.0 (4.9)	9, 9
Diundecyl	DUP		45 ± 11	3.3			
			29 ± 7	13.3			
Ditridecyl	DTDP					51.5 (10.3)	12, 12

[a] Graham (2).
[b] Saeger and Tucker (3).
[c] Urushigawn and Yonezawa (6).
[d] This study.
[e] Two trials.

tions of PAEs to 50-ml portions of sludge and extraction and analysis demonstrated that the percent recoveries were high (mostly >90%).

All PAEs appeared to biodegrade fairly rapidly during the draw-and-fill phase of the experiment. Table 1 presents the 24-h percent mean biodegradation values determined during draw and fill since statistical analysis (Student's *t* test) demonstrated that results were not dependent upon PAE concentration or week of the test. In general, dimethyl, diethyl, di-*n*-butyl, and dihexyl phthalates underwent primary degradation in excess of 90% in 24 h at both the 1- and 3-mg/liter concentrations. All other PAEs exhibited at least 50% primary degradation (loss of parent compound) in 24 h regardless of test concentration.

Data concerning primary biodegradation during the die-away phase are also presented in Table 1. Percent primary biodegradation values presented in Table 1 are not corrected for recovery. All PAEs tested had undergone primary degradation in excess of 90% (as confirmed by two consecutive measurements) before completion of the 19-day die-away period. In fact, under these conditions, dimethyl, diethyl, di-*n*-butyl, and dihexyl phthalates appear to biodegrade in excess of 90% in less than 24 h.

The variability observed between the duplicate samples of each PAE was minimal. Primary biodegradation values obtained in duplicate samples during week 3 of the draw-and-fill phase differed by only 6.8% on the average. The number of days to achieve 90% or greater degradation in duplicate samples (obtained in the die-away phase) differed by an average of only 0.3 days. Only two cases were observed in which duplicate samples of a PAE reached 90% or greater primary biodegradation on different days (di-2-ethylhexyl and heptyl, nonyl, undecyl phthalates).

The combined SCAS and activated sludge die-away procedure is based upon the Soap and Detergent Association (4) SCAS and the static activated sludge die-away method of Zahn and Wellens (8). The SCAS portion of the test was used as an acclimation step for the die-away phase (7). However, with the PAEs, acclimation either is not necessary or occurs so rapidly that, by day 3 of week 1 of exposure to PAE, the 24-h biodegradation rate is very reproducibly high.

The test procedure used worked well and was fairly reproducible for this series of compounds providing dispersion was accomplished with an acetone stock solution. This is recommended for all chemicals that have water solubilities as low as those of the PAEs. The 19-day die-away has been demonstrated to be an extremely vigorous system (1) and, therefore, may give values higher than likely to occur in an activated sludge treatment plant.

Table 1 also provides a comparison of the results from this investigation and those from other activated sludge studies. The PAEs are listed in order of increasing molecular weight. There is a statistically significant ($P < 0.001$) general trend toward decreasing biodegradability with increasing molecular weight for both the 24-h SCAS biodegradability and the 19-day die-away biodegradability, but this may be due to decreased water solubility (Howard et al., submitted) (availability) with increasing molecular weight. Saeger and Tucker (3) found a similar trend. They measured the SCAS 24-h

biodegradability for butyl benzyl, butylglycol butyl, di-2-ethylhexyl heptyl, nonyl, undecyl, and diundecyl phthalates. Of the two PAEs tested by Saeger and Tucker (3) and examined in this study, di-2-ethylhexyl and heptyl, nonyl, undecyl phthalates were both degraded less in the Saeger and Tucker (3) study.

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LITERATURE CITED

1. **Gerike, P., and W. K. Fischer.** 1981. A correlation study of biodegradability determinations with various chemicals in various tests. II. Additional results and conclusions. Ecotox. Environ. Saf. **5**:45–55.
2. **Graham, P. R.** 1973. Phthalate ester plasticizers—why and how they are used. Environ. Health Perspect. **3**:3–12.
3. **Saeger, V. W., and E. S. Tucker.** 1976. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microbiol. **31**:29–34.
4. **Soap and Detergent Association, Subcommittee on Biodegradation Test Methods.** 1965. A procedure and standards for the determination of the biodegradability of alkylbenzene sulfonate and linear alkylate sulfonate. J. Am. Oil Chem. Soc. **42**:986–93.
5. **Sugatt, R. H., D. P. O'Grady, S. Banerjee, P. H. Howard, and W. Gledhill.** 1984. Shake flask biodegradation of 14 commercial phthalate esters. Appl. Environ. Microbiol. **47**:601–606.
6. **Urushigawa, Y., and Y. Yonezawa.** 1979. Chemico-biological interactions in biological purification systems. VI. Relation between biodegradation rate constant of di-*n*-alkyl phthalate esters and their reaction times in reverse phase partition chromatography. Chemosphere **5**:317–20.
7. **U.S. Environmental Protection Agency.** 1979. Toxic Substances Control Act premanufacture testing for new chemical substance. Fed. Regist. **44**:16240–92, 16 Mar.
8. **Zahn, R., and H. Wellens.** 1974. Simple procedure for testing the biodegradability of products and sewage components. Chem. Ztg. **19**:228–32.